CHARACTERISTICS OF A NEW GROUP OF ENTEROPATHOGENIC E. COLI PRODUCING ENTEROTOXIN

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CHARACTERISTICS OF A NEW GROUP OF ENTEROPATHOGENIC E. COLI PRODUCING ENTEROTOXIN

[Paper by T. A. Avdeyeva, Yu. Ye Polatskiy, L. A. Smirnova, Ye. M. Dragunskaya, E. V. Poyasova and V. G. Chalenko; of the Institute of Epidemiology and Microbiology imeny Pastei, the Institute of Experimental Medicine, USSR Academy of Medical Sciences and the Institute of Vaccines and Serums; received by the editors, 15 November 1972. In the periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology). Zh. Mikrobiol. Epid. Immunobiol. 50:11:9-12 (1973)

Progress realized in the past few years in the development of a series of experimental models has greatly advanced existing concepts of the etiology of intestinal infections. Assuring, as they have done, a deeper understanding of the biology and pathenogenic properties of shigellae, salmonellae and enteropathenogenic bacilli, experimental models have proven likewise serviceable in the detection of the ability of bacteria to produce intestinal infection in human beings. Infection of a loop of the small intestine, among other methods, has been used to establish enteropathogenicity (experimental animal, rabbit; 5, 14, 25). The use of this particular model has also favored the knowledge of the pathogenesis of cholera [13, 15, 18] and the enteroxigenic intestinal bacilli (EIB), the causative agents of so-called colibacillosis of swine, cattle, and some other animals [21, 22, 24]. In the past few years, this particular method has been used to observe cholera-like illnesses in both children

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and adults [16, 17, 19, 20].

Enteroxigenic intestinal bacilli (EIB) are known thus far in only a few serological types, distinguished by the O-, K- and H-antigens (O6:H16, O15; H11,O78: K80, O148: H28, etc.). These produce enterotoxins--one thermostable, observed in superfluous culture liquids, the other thermo-labile, contained in cellulary lysates. With use of an isolated small-intestine (rabbit) model, living cultures and their sterile cell-less substrates lead to expansion of the intestinal loops, owing to accumulation of the liquid content. The etiological role of EIB in human gastroenteritis has already been demonstrated [16, 19, 23].

In the USSR a different, the so-called lung model, is being used, for the induction of shigella pneumonia in intranasally infected albino mice [6]. Using a lung model, it is possible, through a microbiological and morphological study, to differentiate various different excitants of intestinal infections [7, 10, 11].

What is intended in the present article is a presentation of the results of a study of intestinal illnesses of uncertain etiology with use of the "lung model" [1-3, 8], which has made possible the discovery of microorganisms of the genus Escherichia which, in distinction from known excitants of intestinal infections, produced death in infected mice within a few hours. The symptoms accompanying death (acute asphyxia, spasms, exudation of serobloody liquid from the nose and mouth) have not been previously observed.

In all, 43 strains of bacteria with the indicated characteristics were studied. On the basis of the O-antigen, these were divided into 12 serological types, of which six fell into O-types 1, 6, 16, 86, 112 a and 115). The total number of possible types was 21.

It was established, as a result of the study, that intranasal infection of mice in the course of 8-15 minutes with cells of a 24-hour bouillon culture is accompanied by multiplication of the exciting agents in lung tissue and by the development of a pathological process distinguished by microbiological and morphological characteristics from a similar intranasal infection of mice with known causative agents of intestinal infections [1-3]. Acute intoxication was the basis of the pathological process. Infection of the animals led to vascular damage and rapid advance of serous-hemorrhagic pulmonary edema. Multiplication of the bacteria took place only in the lumen of the alveolus. As distinct from shigellae and shigella-like enterogenic intestinal bacilli, the bacteria studied did not penetrate, and conjunctival infection of the mice was not accompanied by any development of keratoconjunctivitis [8].

The capability of intestinal bacilli obtained from intestinal diseases of uncertain etiology to produce fatal results in mice against a background of comparable symptoms is regarded, by the authors, as a precise sign of enteropathogenity. This is in fact confirmed by the results of analysis of a case of mass illness in children and adults from whom types 01 and 0112 ab bacilli were abstracted. These particular microorganisms, upon introduction into mice, produced early death in the animals, accompanied by the typical symptoms of acute asphyxia [4, 9, 12].

Analysis of the clinical symptoms and the morphological picture of the process as presented in the mice infected in our experiments leads to the conclusion that these bacteria are the producers of toxins. It is the authors' opinion that the presently used "lung" method, which is in essence a model of

an isolated rabbit small intestine loop, can be used to observe intestinal illnesses anlogous to those described in recent years in the foreign medical literature.

First of all, in order to resolve the questions presented here, it was necessary to determine whether enterotoxins were indeed being produced in the cultures which we studied.

No toxins, were actually discovered in the superfluid liquids of the cultures. Quite different results have been obtained in the study of lysates [18], and intranasal administration to mice of such cell-less substrates obtained from three different strains has shown that these cultures (intestinal bacteria) do indeed produce thermoliabile toxins: all of the test animals to which were administered cellular lysates died. The lysates were completely inavtivated following a thirty-minute period of heating at 60°C. The causes of the differences in the times preceeding death following intranasal administration (1 - 8 hours) of the cultures and of their lysates (21 - 93 hours) are deserving of particular attention. It has been established, however, that there is a great similarity between the pathological condition which arose in the mice infected with the cultures, and their lysates. The intranasal administration to the mice of the thermolabile toxin, just like the infection with the cultures, was accompanied by a marked accumulation of serous-hemorrhagic exhudate in the pulmonary tissues. Quite obviously, the basic mechanism involved in the action of the bacteria which we studied was associated with their toxigenicity.

The propesition which we make here concerning the essential similarity between the lung model and the model of an isolated small intestinal loop of a rabbit was indeed confirmed by further parallel tests run on these same models

studied by the authors and prepared by them from sterile cell lysates.

Upon introduction of six strains and their thermolabile lysate (2 strains), we obtained positive reactions together with distension of the loopes, owing to the accumulation of fluid (5-18 ml).

In this way, it was possible to study the capability of the cultures and of the product of thermolabile toxin on the basis of the two models tested.

The possibility of determining (EIB) with use of a lung model is of rather considerable importance, inasmuch as the model suggested by De and Chatterjee [14] is more complex than the method of intrnasal administration to albino mice.

The data obtained expand existing concepts of enteropathegenic intestinal bacilli. Further study of the biologic properties of the enterotoxigenic Escherichieae along with clinical study and the pathogenesis of the associated illnesses, is of particular importance in connection with the necessity of differentiating between these diseases and clinically similar forms of cholera.

CONCLUSIONS

1. It was established that the death of mice due to acute serous-hemorragic pulmonary edema during the first few hours following intranasal infection with cultures of intestinal bacilli derived from sick animals suffering from intestinal disease of undetermined origin, resulted from their enterotoxigenicity.

2. We have demonstrated the possibility of showing the presence of enterotoxic intestinal bacilli and of establishing their capability for the production of thermolabile toxin on a lung model; this is a simpler and more convenient method than those regularly used for this purpose.

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A possibility of using a pulmonary model, more simple and accessible in comparison with other models, for detection of enterotoxigenic E. coli and establishment in them of the capacity to production of a thermolabile toxin was shown. An enterotoxin which in living cultures caused in intranasally infected albino mice serous-hemorrhagic edema of the lungs and subsequent death of the animals was obtained. Enterotoxigenicity of cultures was confirmed on a model of an isolated loop of rabbit small intestine.

...mebacteria, nontoxigenic and starch fermenting (circulating in the USSR) referred accord... to the acting instruction—on the basis of morphological, cultural and biochemical signs ... C. diphtheriae of gravis type. Eleven sera to the known serological types of C. diphtheria ... also 2 sera to bacteriophage types K and ABCDfg were used for typing. A total of ... strains, representatives of 12 bacteriophage types, isolated from 152 carriers in 93 foci ... populated localities of 23 regions in the USSR in 1963—1969 were divided into 2 groups. ... first group included all (without exception) strains of ABCDFGH, ABCDFG, ABCDF, ...CD, ABD, ACD, AF, A, CDf bacteriophage types spontaneously agglutinating in 3% NaCl ...tion on a buffer, stabilizing in a 0.2% twin 80 solution, agglutinating (in titres of 1 : 200— ...800) by sera of 9 and 11 serological types and by the serum of ABCDfg bacteriophage ...pe. It was formerly demonstrated that these bacteriophage types were capable of converting ...toxigenic in case of infection with toxi-viruses, in connection with which they were referred ... the true ...revealed in 31.6% ...of the carri... own true C. diph... ...teriae stra... the strain of this ...bacteriopha... ses were referred ...to the secon... 3% of the carrier ...foci with ...

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ХАРАКТЕРИСТИКА НОВОЙ ГРУППЫ ЭНТЕРОПАТОГЕННЫХ КИШЕЧНЫХ ПАЛОЧЕК, ПРОДУЦИРУЮЩИХ ЭНТЕРОТОКСИН[1]

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Успехи, достигнутые за последние годы в разработке ряда экспериментальных моделей, во многом способствовали расширению существующих представлений об этиологии кишечных инфекций. Обеспечивая возможность более углубленного изучения биологии и патогенетических свойств шигелл, сальмонелл, энтеропатогенных кишечных палочек (ЭПКП), экспериментальные модели оказались пригодными и для раскрытия у бактерий способности вызывать кишечную инфекцию у людей (энтеропатогенность). Для установления энтеропатогенности наряду с другими способами используется заражение петли тонкой кишки кролика [5, 14, 25]. Использование этой модели способствовало изучению патогенеза холеры [13, 15, 18], развитию учения об энтеротоксигенных кишечных палочках (ЭТКП) — возбудителях так называемого колибациллеза свиней, телят и других животных [21, 22, 24]. В последние годы при помощи того же метода обнаружены ЭТКП — возбудители холероподобных заболеваний детей и взрослых [16, 17, 19, 20].

ЭТКП представлены пока еще небольшим рядом серологических типов, различающихся по О-, К- и Н- антигенам (О6 : Н16, О15 : Н11, О78 : К80, О145 : Н28 и пр.). Они продуцируют энтеротоксины — термостабильный, обнаруживаемый в надосадочных жидкостях культур, и термолабильный, содержащийся в клеточных лизатах. На модели изолированной петли тонкой кишки кролика живые культуры и их стерильные бесклеточные субстраты вызывают расширение кишечных петель за счет скопления в них жидкого содержимого. Доказана этиологическая роль ЭТКП при гастроэнтерите у людей [16, 19, 23].

В нашей стране для тех же целей используется другая, так называемая легочная модель, предложенная для воспроизведения шигеллезной пневмонии у интраназально зараженных белых мышей [6]. На легочной модели по количественной микробиологической и морфологической характеристике

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